

# Exogenous Phytase Plus Cellulase and Phosphorus Excretion in Lactating Dairy Cows

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## Abstract

The objectives were to assess the effects of exogenous phytase plus cellulase on P excretion in lactating cows. The effects of an exogenous phytase plus cellulase mixture and dietary P content on P partitioning and excretion were evaluated in nine early lactation cows (mean = 27 d in milk); six of the cows were ruminally cannulated. Cows were assigned to treatments in replicated (three) 3 × 3 Latin squares, and each cow received each treatment sequentially in three, 21-d periods. Diets were 45% forage (all corn silage) and included supplemental P (high P; 0.47%), no supplemental P (low P; 0.32%), or no supplemental P with exogenous phytase (low P-enzyme; 0.32%). Total collection of milk, urine, and feces was conducted on d 19 to 21 of each period. There were no effects of dietary P or exogenous phytase plus cellulase on DMI, milk yield, or milk composition. Excretion of feces was unaffected by diet, but urine excretion was less by cows fed the low P diets than by cows fed the high P diets (16.5 vs 21.3 kg/d). Compared with cows fed high P diets, cows fed the low P diets had reduced P intake (68.1 vs 103.9 g/d), reduced fecal (34.4 vs 51.3 g/d) and urinary P excretion (2.8

vs 9.2 g/d), and lesser P balance (–8.0 vs. 4.4 g/d). The addition of exogenous phytase plus cellulase did not affect P intake, milk P, fecal P, or urinary P excretion, but apparent P digestibility tended to be greater in cows fed diets supplemented with the enzyme formulations (50.1% vs 40.5% for low P-enzyme and low P, respectively).

(Key Words: Phosphorus Excretion, Phytase, Lactating Cows.)

## Introduction

The development of nutritional strategies to reduce P excretion by livestock is an important aspect of long-term efforts to reduce P loading to surface water. The availability of P in feedstuffs affects P excretion, but assumptions of availability of feed P are based on relatively few studies (Young et al., 1966; Dayrell and Ivan 1989; Martz et al., 1990; Martz et al., 1999). Improved P availability from feed would allow the tissue-level needs of the animal to be met with reduced P intake, thus reducing the P content of livestock manure. The development of phytase additives for monogastric animals is one example of nutritional manipulation of P availability and excretion. The endogenous phytase activity provided by ruminal microorganisms makes the P in grains and forages more available to

ruminants than to non-ruminants (Clark et al., 1986; Morse et al., 1992b). Because P intake and excretion are so tightly linked (Morse et al., 1992a; Wu et al., 2000; Knowlton and Herbein, 2002), even small improvements in availability of feed P would reduce P excretion significantly. For instance, improving P availability of dairy rations by 5 percentage units (i.e., from 60 to 65%) and reducing P intake accordingly to keep absorbed P constant would reduce P excretion by dairy cows by 15%, thus reducing the potential loading to surface water significantly (Knowlton et al., 2004).

There is some evidence in the literature that the endogenous phytase activity of the ruminal microorganisms varies with diet. Yanke et al. (1998) observed that, in vitro, strains of *Selemonas ruminantium*, a starch-digesting bacterium, had substantial phytase activity and that phytase activity of mixed ruminal fluid was greater in steers fed a barley-based diet than in those fed an all-hay diet. In a continuous culture fermenter utilizing ruminal fluid from goats, Godoy and Meschy (2001) observed an increase in phytate P availability with an organic P buffer compared with an inorganic P buffer. Guyton et al. (2003) observed an interaction of starch source and supplementation with purified phytic acid on endogenous ru-

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minimal phytase activity. The direction of the response to phytic acid supplementation differed with starch source. In cows fed diets containing dried ground corn, ruminal phytase activity was numerically greater in cows fed phytic acid than in cows fed the low P diet. In contrast, in cows fed diets based on steam-flaked corn, phytase activity was similar with and without phytic acid supplementation (Guyton et al., 2003).

These studies, which indicate variation in endogenous ruminal phytase activity, suggest opportunity to improve P availability with exogenous phytase under specific dietary conditions. The objective was to evaluate the effects of exogenous phytase plus cellulase on P excretion in lactating cows.

## Materials and Methods

**Cows and Diets.** Nine early lactation cows (six ruminally cannulated;  $27.2 \pm 10.4$  d in milk) were fed diets containing 0.32 or 0.47% P (~70 and 120% of NRC, 2001). The low P diets were fed with or without the addition of fibrolytic and phytase enzyme formulations. The fibrolytic enzyme formulation was a commercial preparation from fungal extracts, with 15,000 units of cellulase activity/g (Animal Feed Technologies, Greeley, CO); one unit was defined as the cellulase activity that produced a relative fluidity change of 1.0 in 100 min in a 0.2% (wt/vol) sodium carboxymethyl cellulose (CMC type 7HP; Hercules, Inc., Wilmington, DE) solution under assay conditions (pH 4.5 and 40°C) as measured with a Size 100 Calibrated Cannon-Fiske type viscosimeter. The phytase formulation contained 5000 units of phytase activity/g; one was unit defined as the phytase activity that released 1.0  $\mu$ mol of phosphate/min under assay conditions (pH 5.5 and 37°C).

The granular enzyme formulations were mixed with a corn grain carrier, and the enzyme-corn grain mixture or control (an equal quantity of corn grain containing no enzyme formula-

**TABLE 1. Ingredient composition of diets.**

Item	High P	Low P	
		(% of diet DM)	
Corn silage	44.9	45.0	45.0
Corn grain	26.5	26.6	26.6
Soybean meal	14.6	14.7	14.7
Expeller soybean meal <sup>a</sup>	1.36	1.36	1.36
Calcium carbonate	1.40	1.89	1.88
Vitamin-mineral mix <sup>b</sup>	9.19	9.22	9.21
Dicalcium phosphate	0.81	0.00	0.00
Urea	0.52	0.52	0.52
Sodium bicarbonate	0.52	0.52	0.52
Salt	0.19	0.19	0.19
Enzyme formulation <sup>c</sup>	0.00	0.00	0.04

<sup>a</sup>Soyplus™ (West Central Soy, Ralston, IA).

<sup>b</sup>Each kilogram contained 1462 mg of Ca, 1758 mg of P, 11,100 mg of Mg, 1270 mg of S, 3.45 mg of Co, 136 mg of Cu, 6.8 mg of I, 170 mg of Mn, 2.64 mg of Se, 340 mg of Zn, 46,900 IU of vitamin A, 16,960 IU of vitamin D, and 256 IU of vitamin E.

<sup>c</sup>Each tonne contained 200 g of fibrolytic enzyme formulation (15,000 units cellulase activity/g) and 280 g of phytase (5000 units phytase activity/g).

tion) was added to the grain portion of the diet prior to mixing of the TMR (200 g of fibrolytic enzyme formulation and 280 g of phytase/tonne DM fed). Ingredient and nutrient composition of diets are presented in Tables 1 and 2. This experiment was conducted with approval from the Virginia Tech Animal Care Committee.

**Experimental Design and Sampling.** Cows were grouped by previous lactation mature-equivalent milk yield and assigned to one of three,  $3 \times 3$  Latin squares. Squares were balanced for residual effects. Each experimental period lasted 21 d. Cows were fed in Calan doors for the first 17 d of each period and were moved to individual stalls on d 18 for total collection of feces, urine, and milk. Cows were fed once daily at 0800 h and milked at 0700 and 1900 h. Feed was offered at 5 to 10% in excess of previous day's intake (wet basis).

On d 18, a sterile Foley urine catheter (22 French, 75 cc; C. R. Bard, Inc., Covington, GA) was inserted into the urethra for total collection of urine. All excreted urine, feces, and milk were collected on d 19, 20, and 21.

Urine was weighed at 4-h intervals, acidified to pH <2 (22 mL of 6N HCl/kg urine), pooled, subsampled after 24 h, and stored frozen for later analysis. All excreted feces were collected at 4-h intervals and stored in a sealed container, then weighed, thoroughly mixed, and subsampled daily. Feed ingredients (forages and concentrates) were sampled once each week, and orts were weighed and sampled daily. On d 19, 20, and 21, feed offered and refused was measured, total milk weights were recorded, and milk was sampled at six consecutive milkings.

**Laboratory Analysis.** Samples of feed ingredients and orts were dried to constant weight at 60°C in a forced-air drying oven (Wisconsin Oven, Memmert; Schwabach, Germany). Dried samples were ground through a 1-mm screen in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA). Feed and ort samples were analyzed in duplicate for N, P, Ca, ash (AOAC, 1990), and NDF and ADF sequentially with  $\alpha$ -amylase (Van Soest et al., 1991). Feces and urine samples were analyzed for P (AOAC, 1990) and feces samples for NDF as indicated previously. Milk samples were

TABLE 2. Nutrient composition of diets.

Item	High P	Low P	Low P-enzyme	SEM	P <		
					Treatment	Dietary P <sup>a</sup>	Enzyme <sup>b</sup>
	( % of dietary DM )						
CP	17.4	17.1	17.4	0.35	0.75	0.69	0.53
ADF	14.1	14.2	14.1	0.06	0.55	0.41	0.49
NDF	26.1	26.1	26.0	0.16	0.83	0.73	0.62
Ash	8.73	9.02	8.91	0.03	0.80	0.55	0.81
Ca	1.11	0.99	0.92	0.07	0.25	0.13	0.48
P	0.47	0.32	0.32	0.01	0.01	0.01	0.87

<sup>a</sup>High P vs low P.

<sup>b</sup>Low P vs low P enzyme.

analyzed for fat, protein, total solids, SNF (Dairy Herd Improvement Association, Blacksburg, VA), and P (AOAC, 1990). Retention, milk output, and excretion of P were calculated.

**Statistical Analysis.** All data were analyzed using the MIXED procedure of SAS® (SAS Institute, Cary, NC) with the model

$$Y_{ijkl} = \mu + s_i + c_j(s)_i + D_k + T_l + e_{ijkl}$$

where

$\mu$  = overall mean,  
 $s_i$  = random effect of square  
 (i = 1 to 3),

$c_j(s)_i$  = random effect of cow within square (j = 1 to 3),

$D_k$  = fixed effect of period  
 (k = 1 to 4),

$T_l$  = fixed effect of treatment  
 (l = 1 to 3), and

$e_{ijkl}$  = residual error.

Residual error was used to test the main effect of treatment, and pre-planned contrasts were used to evaluate the effect of dietary P (high P vs low P and low P-enzyme) and phytase plus cellulase addition (low P vs low P-enzyme). Differences were declared significant at  $P < 0.05$ , and trends were declared at  $P < 0.10$  un-

less otherwise indicated. Results are reported as least squares means.

## Results and Discussion

**Nutrient Composition of Diets.** Ingredient and nutrient composition of treatment diets in Experiment 2 are presented in Tables 1 and 2. As planned, diets differed only in P content.

**P Intake, Digestion, and Excretion.** Compared with cows fed high P, cows fed the low P diets had reduced P intake (68.1 vs 103.9 g/d; Table 3), reduced fecal (35.8 vs 51.3 g/d)

TABLE 3. Effects of dietary P and addition of exogenous phytase plus cellulase on P intake and partitioning in lactating Holstein cows.

Item	High P	Low P	Low P-enzyme	SEM	P <		
					Treatment	Dietary P <sup>a</sup>	Enzyme <sup>b</sup>
P Intake, g/d	103.9	66.7	69.5	5.36	0.01	0.01	0.66
Fecal P excretion, g/d	51.3	38.0	33.6	4.46	0.02	0.01	0.41
Apparent P digestibility, %	46.6	40.5	50.1	4.47	0.25	0.80	0.11
Urinary P, g/d	5.42	1.87	1.20	1.47	0.03	0.01	0.65
Total P excretion, g/d	56.5	40.0	34.8	3.81	0.01	0.01	0.32
Ruminal phytase activity, nmol Pi released/min per mL rumen fluid	24.0	22.2	18.8	4.65	0.69	0.89	0.41
Milk P, g/d	34.3	33.2	35.4	2.20	0.58	0.97	0.31
Milk P, % of P intake	34.9	51.7	51.3	3.2	0.01	0.01	0.91
P Balance, g/d	8.28	-8.82	-4.58	3.33	0.01	0.01	0.33

<sup>a</sup>High P vs low P.

<sup>b</sup>Low P vs low P-enzyme.

**TABLE 4. Effects of dietary P and addition of exogenous phytase plus cellulase on feed intake, digestibility, and manure excretion in lactating Holstein cows.**

Item	High P	Low P	Low P-enzyme	SEM	<i>P</i> <		
					Treatment	Dietary P <sup>a</sup>	Enzyme <sup>b</sup>
DMI, kg/d	22.2	21.5	21.7	1.35	0.89	0.65	0.87
Apparent DM digestibility, %	72.7	72.1	73.2	1.52	0.78	0.99	0.49
Fecal excretion, kg/d DM	5.78	5.98	5.80	0.53	0.91	0.82	0.71
Fecal excretion, kg/d wet	37.8	38.8	37.1	3.61	0.85	0.97	0.57
Urine output, kg/d	21.3	15.5	17.5	1.43	0.01	0.01	0.13

<sup>a</sup>High P vs low P.<sup>b</sup>Low P vs low P-enzyme.

and urinary P excretion (1.5 vs 5.4 g/d), and lesser P balance (-6.7 vs 8.3 g/d). Others have observed similar reduced fecal and urinary P excretion with decreased dietary P intake (Wu et al., 2000; Knowlton et al., 2001; Wu et al., 2001; Knowlton and Herbein, 2002). Apparent P digestibility was unaffected by dietary P content, similar to the observations of Guyton et al. (2003). Milk P secretion as a proportion of P intake was greater in cows fed the low P diets than in cows fed the high P diets (51.5% vs 34.9%). Morse et al. (1992a), Knowlton et al. (2001), and Knowlton and Herbein (2002) also observed that milk P as a percentage of P intake decreased with an increase in dietary P content, as the P content of milk is relatively constant.

Addition of exogenous cellulase and phytase did not affect P intake, milk P, fecal P, or urinary P excretion (Table 3), but apparent P digestibility tended to be greater in cows supplemented with exogenous enzymes (50.1% vs 40.5% for low P-enzyme and low P, respectively;  $P < 0.11$ ). This trend was due to a slight, non-significant, increase in P intake (+2.8 g/d) combined with a numerical decrease in fecal P excretion (-5.4 g/d) with phytase supplementation. Most published studies have reported that ruminal phytase activity does not limit digestion of dietary P (Reid and Franklin, 1947; Clark et al., 1986; Morse et al., 1992b). However, there is some evidence of incomplete digestion of phytic acid in ruminants. Hill et al. (2002) reported that low phytic acid

corn, but not exogenous phytase, reduced fecal P concentration in midlactation cows. Interpretation of that study is problematic, as the low phytic acid corn was not isogenic with the normal corn. Duskovala et al. (2001) observed measurable phytic acid in the feces of grain-fed, weaned calves at 6 and 13 wk of age, indicating incomplete digestion.

An alternative explanation for the effect of the supplementation with exogenous enzymes on apparent P digestibility is increased digestion of the entire diet caused by the cellulase in the formulation. The lack of effect of enzyme addition on apparent DM digestibility (Table 4) does not support this explanation. Additional work is needed to clearly separate any effects of the two enzymes and to

**TABLE 5. Effects of dietary P and addition of exogenous phytase plus cellulase on lactational performance in lactating Holstein cows.**

Item	High P	Low P	Low P-enzyme	SEM	<i>P</i> <		
					Treatment	Dietary P <sup>a</sup>	Enzyme <sup>b</sup>
Milk yield, kg/d	38.7	38.8	41.3	2.34	0.45	0.51	0.30
Milk fat, kg/d	1.10	1.16	1.25	0.09	0.25	0.19	0.33
True protein, kg/d	1.09	1.06	1.13	0.95	0.85	0.95	0.57
Lactose, kg/d	1.84	1.83	1.96	0.13	0.49	0.59	0.30
SNF, kg/d	3.30	3.27	3.48	0.22	0.56	0.70	0.33
Milk urea N, mg/dL	16.6	15.3	16.2	1.2	0.51	0.38	0.43

<sup>a</sup>High P vs low P.<sup>b</sup>Low P vs low P-enzyme.

evaluate varying doses of exogenous phytase in different basal diets.

**Feed Intake, Manure Production, and Milk Yield.** Intake and digestibility of DM and excretion of feces were unaffected by diet (Table 4), but urine excretion was less by cows fed the low P diets than by cows fed the high P diet (16.5 vs 21.3 kg/d). Only one other experiment has reported an effect of dietary P content on urine excretion. Burkholder et al. (2004) observed that cows fed supplemental purified phytic acid excreted more urine (+1.9 kg/d) than cows fed low P diets. The effect of dietary P on urine excretion is likely an indirect effect, but, in the absence of water consumption data, the biological mechanism is unclear. Diets were formulated to contain the same Na and K content, with the same quantities of salt and sodium bicarbonate provided to all cows.

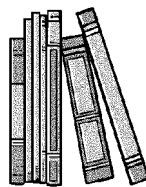
Neither dietary P content nor exogenous phytase plus cellulase affected milk yield (39.6 kg/d) or milk composition (Table 5). Milk fat content was low ( $\leq 3\%$  or less), reflecting the relatively low forage content (45%) and the use of corn silage as the sole source of forage.

## Implications

Reduced dietary P reduced P excretion and improved capture of dietary P in milk. Addition of exogenous phytase plus cellulase to the low P diet tended to improve apparent P digestibility. Additional work is needed to clearly separate the effects of the two enzymes, to evaluate varying doses of exogenous phytase in different basal diets, and to evaluate effects of low P diets with and without phytase on P balance throughout lactation. Utilization of exogenous enzyme formulation in conjunction with reduced dietary P may improve producers' ability to meet P-based nutrient management regulations.

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## Literature Cited

- Association of Official Analytical Chemists. 1990. Official Methods of Analysis. (15th Ed.). AOAC, Arlington VA.
- Burkholder, K. M., A. D. Guyton, J. M. McKinney, and K. F. Knowlton. 2004. The effect of steam flaked or dry ground corn and supplemental phytic acid on nitrogen partitioning in lactating cows and ammonia emission from manure. *J. Dairy Sci.* 87:2546.
- Clark, W. D. J., J. E. Wohlt, R. L. Gilbreath, and P. K. Zajac. 1986. Phytate phosphorus intake and disappearance in the gastrointestinal tract of high producing dairy cows. *J. Dairy Sci.* 69:3151.
- Dayrell, M. S., and M. Ivan. 1989. True absorption of phosphorus in sheep fed corn silage and silage supplemented with dicalcium or rock phosphate. *Can. J. Anim. Sci.* 69:181.
- Duskova, D., R. Dvorak, V. Rada, J. Doubek, and M. Marounek. 2001. Concentration of phytic acid in faeces of calves fed starter diets. *Acta Vet. Brno.* 70:381.
- Godoy, S., and F. Meschy. 2001. Utilization of phytate phosphorus by rumen bacteria in a semi-continuous culture system (Rusitec) in lactating dairy goats fed on different forage to concentrate ratios. *Reprod. Nutr. Dev.* 41:259.
- Guyton, A. D., J. M. McKinney, and K. F. Knowlton. 2003. The effect of steam flaked or ground corn and supplemental phytic acid on ruminal phytase activity and P balance in lactating cows. *J. Dairy Sci.* 86:3972.
- Hill, B. E., S. L. Hankins, J. F. Kearney, J. D. Arseneau, D. T. Kelly, S. S. Donkin, B. T. Richter, and A. L. Sutton. 2002. Effects of feeding low phytic acid corn and phytase on phosphorus balance in lactating dairy cows. *J. Dairy Sci.* 85 (Suppl. 1):44.
- Knowlton, K. F., and J. H. Herbein. 2002. Phosphorus balance during early lactation in dairy cows fed diets varying in phosphorus content. *J. Dairy Sci.* 85:1227.
- Knowlton, K. F., J. H. Herbein, M. A. Meister-Weisbarth, and W. A. Wark. 2001. Nitrogen and phosphorus partitioning in lactating Holstein cows fed different sources of dietary protein and phosphorus. *J. Dairy Sci.* 84:1210.
- Knowlton, K. F., J. S. Radcliffe, C. L. Novak, and D. A. Emmerson. 2004. Animal management to reduce phosphorus losses to the environment. *J. Anim. Sci.* 82E:173.
- Martz, F. A., A. T. Belo, M. F. Weiss, and R. L. Belyea. 1990. True absorption of calcium and phosphorus from alfalfa and corn silage fed to lactating cows. *J. Dairy Sci.* 73:1288.
- Martz, F. A., A. T. Belo, M. F. Weiss, and R. L. Belyea. 1999. True absorption of calcium and phosphorus from corn silage fed to nonlactating, pregnant dairy cows. *J. Dairy Sci.* 82:618.
- Morse, D., H. H. Head, and C. J. Wilcox. 1992b. Disappearance of phosphorus in phytate from concentrates in vitro from rations fed to lactating dairy cows. *J. Dairy Sci.* 75:1979.
- Morse, D., H. Head, C. J. Wilcox, H. H. V. Horn, C. D. Hissem, and B. Harris, Jr. 1992a. Effects of concentration of dietary phosphorus on amount and route of excretion. *J. Dairy Sci.* 75:3039.
- National Research Council. 2001. Nutrient Requirements of Dairy Cattle. (7th Rev. Ed.). Natl. Acad. Sci., Washington, DC.
- Reid, R. L., and M. C. Franklin. 1947. The utilization of phytate phosphorus by sheep. *The Austr. Vet. J.* 25:136.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583.
- Wu, Z., L. D. Satter, A. J. Blohowiak, R. H. Stauffacher, and J. H. Wilson. 2001. Milk production, estimated phosphorus excretion, and bone characteristics of dairy cows fed different amounts of phosphorus for two or three years. *J. Dairy Sci.* 84:1738.
- Wu, Z., L. D. Satter, and R. Sojo. 2000. Milk production, reproductive performance, and fecal excretion of phosphorus by dairy cows fed three amounts of phosphorus. *J. Dairy Sci.* 83:1028.
- Yanke, L. J., H. D. Bae, L. B. Selinger, and K. J. Cheng. 1998. Phytase activity of anaerobic ruminal bacteria. *Microbiology* 144:1565.
- Young, V. R., G. P. Lofgreen, and J. R. Luick. 1966. The effects of phosphorus depletion, and of calcium and phosphorus intake, on the endogenous excretion of these elements by sheep. *Br. J. Nutr.* 20:795.